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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Bruck, et al.

August 16, 2001

Serial No.: 09/509,239

Group Art Unit No.: 1648

Filed: March 23, 2000

Examiner: U. Winkler

For: "Fusion Proteins Comprising HIV-1 Tat and/or Nef Proteins"

Assistant Commissioner for Patents
Attn: Chief Draftsman
Washington, D.C. 20231

TRANSMITTAL OF FORMAL DRAWINGS

In response to the Notice of Informal Drawings mailed on July 17, 2000 attached
please find:

(a) the formal drawings for this application with each sheet indicating the Serial
Number and the Group Art Unit. Number of Sheets: 18

(b) a copy of the Notice of Informal Drawings.

Respectfully submitted,

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FIG. 1A Map of plasmid pRIT14586

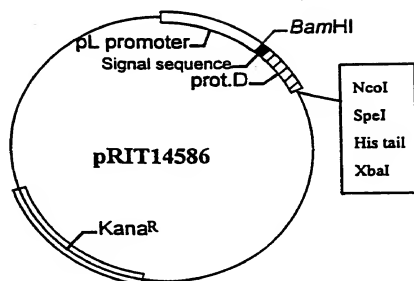


FIG. 1B Coding sequence of the first 127 amino acids of protein D and multiple cloning site. The signal sequence is underlined.

BamHI
 ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTA CTA GCA GGT TGT AGC AGC
 Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser
 CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT
 His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly
 GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT
 Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln Ala
 GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC
 Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His
 TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT
 Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg
 TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA
 Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu
NcoI SpeI XbaI
 ACC ATG GCC ACG TGT GAT CAG AGC TCA ACT AGT GGA CAC CAT CAC CAT CAC CAT TAA TCT AGA
 Thr Met Ala Thr Cys Asp Gln Ser Ser Thr Ser Gly His His His His His His *

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid of sequence of Figure 1 relates to Seq. ID no. 6.

FIG. 2A

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCAACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAGAACACCAGCTTGTACACCCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGGKWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWI
YHTQGYFPDQNYTPGPGVRYPLTFGWYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLWRFSRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAGCAACCCACCTCCCAA

FIG. 2B

TCCCGAGGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLPEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVLSLKQPTSQSRGDPGTGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCTGTGAGCCTGCAT
GGAATGGATGACCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCCTAGCA
TTTCATCAGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAGTGCCTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCT
TGTACCAATTGCTATTGTAAGAAAGTGTGCTTTCATTGCCAAGTTTGTTCATAACA
AAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 13)

~ ~
MGGKWSKSSVVGWPTVRMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDQNYTFPGVGRYPLTFGWICYKLVPEPDKVEEANKGENTSLHPVSLH
GMDDPEREVLWRFDSRLAFHHVARELHPEYFNKCTSEPVDPRLPEPWKHPGSQPKTA
CTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVLSLKQPTSQSR
GDPGTGPKETSGHHHHHH.

E.coli-expressed c nstructs (fusion constructs)

⇒ LipoD-Nef-HIS

FIG. 2C

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTAGCAATGACTAAGGATGGT
CGTTTAGTGGTTATTACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
TTCCCAATCGTCACTCGTAAAGATGGCGGTTACTATGTCATCGACTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
AGTAGCAATCAGCAGCTACCAATGCTGCTTGTGCTTGGCTAGAAGCAACAAGAGGAG
GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTTAAAAAGAAAAGGGGGGACTGGAAGGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCAACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTT
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACCACAGCTTGTACACCCTGTGAGCCTGCATGGAATGGATGACCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCAGTGGCCCCGA
GAGCTGCATCCGGAGTACTTCAAGAAGTGCATAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
GRLVVIHDHFLDGLTDVAKFPHRHRKDGRIYVIDFTLKEIQSLEMTENFETMGGKW
SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSNTAATNAACAWLEAQE
EEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGLEGLIHSQRRQDILDWIVHTQG
YFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLHGMDPD
EREVLEWRFDLSRLAFHHVARELHPEYFNKCTSGHHHHHH.

⇒ LipoD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 16)

FIG. 2D

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
 AGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATATT
 GCTCACCGTGGTGCTAGCCGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
 GCGTTTGCACAAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
 CGTTTAGTGTTATTACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAA
 TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
 GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCTGGCTAGAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTAAAGAAAAGGGGGGACTGGAAGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCTGATTTGGCAGAACTACACACAGGGGCCAGGGGTGAGATATCCACTGACCTTT
 GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
 GGAGAGAACACCCAGCTTGTACACCCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
 AGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCAGTGGCCCGA
 GAGCTGCATCCGGAGTACTTCAAGAACTGCCTAGTGAAGCAGTAGATCCTAGACTA
 GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGT
 AAAAAGTGTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCC
 TATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
 CAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCAGGGGACCGACAGGCCCG
 AAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
 GRLVVIHDHFLDGLTDVAKFPHRHRKDRYYVIDFLKEIQSLEMENFETMGGKW
 SKSSVVGWPTVVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEFVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWCYKLVPVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFDLSRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCT
 CKKCCFHCQVCFITKALGISYGRKKRRRPPQGSQTHQVSLSKQPTSQSRGDPGT
 PKETSGHHHHHH.

FIG. 2E

⇒ *ProtD-Nef-HIS*

DNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATT**CATCAA**TATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACC**GTGGT**GCTAGCGGTTATTTACCAGAGCATACGTTAGAACTC
AAAGCACTTGC**GTTTG**CACAACAGGCTGATTATTTAGACGAAGATTTAGCAATGACT
AAGGATGGT**CGTTT**AGTGGTTATTCACGACTCTTTTAGATGGCTTGACTGATGTT
GCGAAAAAAT**TCC**CACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAGAAAT**TCAA**AGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCTCGGTAGAAAGCA
CAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAGGGGGGACTGGAA
GGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAGAACCACAGCTTGTTACACCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCAC
GTGGCCCCGAGAGCTGCATCCGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYL
EQDLAMTKDGR**LVVI**HDHFLDGLTDVAKK**FP**HRHRKDGRYYVIDFTLK
EIQ**S**LEM**T**ENFETMG**G**KWSKSSVVGWPTVRERMRRAEPAADGVGAASRDL
EKHGAITSSNTAATNAACA**W**LEAQEE**EE**VGFVTPQVPLRPM**TY**KA**AV**DL**SH**
FLKEKG**G**LEGL**HS**QRRQDIL**DL**W**Y**HTQGYFPD**W**QNYTPGPGVRYPLTFGW
CYKLVPVEPD**K**VEEANKGENTSLLHPVSLHGMD**DP**EREVLEWR**FD**SRLAFH
HVARELHPEYFK**NC**TSGHHHHH.

⇒ *ProtD-Nef-Tat-HIS*

DNA sequence (Seq. ID. No. 20)

FIG. 2F

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATATTGCTCACCCTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTCGCTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTTCGTTTGGTTTATTTCACGATCACTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCAACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAAGAAAGGGGGGACTGGAA
GGGCTAATTCCTCCCAACGAAGACAAGATCCTTGATCTGTGGATCTACCAACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCCAGGGCCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCCTGTGAGCCTGCATGGAATGGAT
GACCTTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAGTGCCTAGTGTGAGCCAGTAGAT
CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAAGCCTAAAACTGCTTGTATCAAT
TGCTATTGTAAAAAGTGTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTA
GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
ACAGGCCCAAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMT
KDGRLLVVIHDHFLDGLTDVAKKFFPHRRKDGRIYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVRERMRRAEPADGVGAASRDLEKHGAITSNTAATNAACAWLEA
QEEEEVGFPTVQVPLRPMTYKAAVDLSHFLKEKGGLIHSQRRQDILDWIIYHT
QGYFPDQNYTPGPGVRYPLTFGWICYLVPVEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLWRFDSRLAFHHVARELHPEYFKNCTSEPVDPRLFPWKHPGSQPKTACTN
CYCKKCCPHQCVCFTIKALGISYGRKKRRQRRRPQGSQTHQVLSLKQPTSQSRGDP
TGPKETSGHHHHH.

≡ Tat-MUTANT-HIS

DNA sequence (Seq. ID. No. 22)

FIG. 2G

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATC	40
CAGGAAGTCAGCCCTAAAACTGCTTGTACCAATTGCTATTG	80
TAATAAGTGTGCTTTTCATTGCCAAGTTTGTTCATAACA	120
GCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGAC	160
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGT	200
TTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG	240
CCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATC	280
ACCATTAA	288

Protein sequence(Seq. ID. No. 23)

Mutated amino-acids in Tat sequences are in bold.

MEPVDPRLEPWKHPGSQPKTACTNICYCKKCCFHCQVCFIT	40
AALGISYGRKKRRQRPRPQGSQTHQVSLSKQPTSQSKGE	80
PTGPKETSGHHHHHHH.	95

⇒Nef-Tat-Mutant-HIS

DNA sequence(Seq. ID. No. 24)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGC	40
CTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC	80
AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACAT	120
GGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTG	160
CTTGTGCCTGGCTAGAAGCACAGAGGAGGAGGAGGTGGG	200
TTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACT	240
TACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAGAA	280
AGGGGGGACTGGAAGGGCTAATTCACCTCCCAACGAAGACA	320
AGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC	360
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCA	400
GATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACC	440
AGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAG	480
AACACCAGCTTGTACACCCTGTGAGCCTGCATGGAATGG	520
ATGACCCCTGAGAGAGAAGTGTGTAGAGTGGAGGTTTGACAG	560
CGCCTAGCATTTTCATCAGTGGCCCGAGAGCTGCATCCG	600
GAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTA	640
GACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAA	680
TGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTTTCAT	720
TGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCT	760
ATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCTCTA	800
AGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC	840
ACCTCCCAATCCAAAGGGGAGCCGACAGGCCCGAAGGAAA	880
CTAGTGGCCACCATCACCATCACCATTAA	909

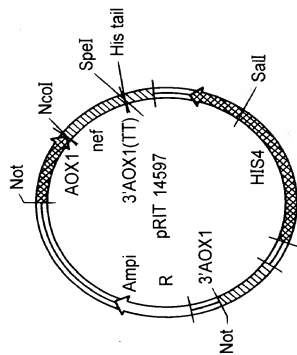
FIG. 2H

Protein sequence (Seq. ID. No. 25)

Mutated amino-acids in Tat sequence are in bold.

MGGKWSKSSVVGWPTVRERMRRAPAADGVGAASRDLEKH	40
GAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMT	80
YKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIIYHTQGY	120
FPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGE	160
NTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHP	200
EYFKNCTSEPVDPRLEPWKHGPSQPKTACTNCYCKKCCFH	240
CQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQP	280
TSQSKGEPTGPKETSGHHHHHH.	302

FIG. 3 Map of pRIT14597 integrative vector

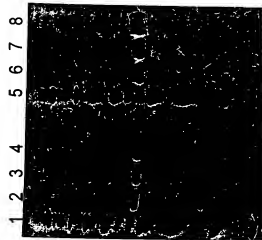


MCS POLYLINKER: nef gene inserted between NcoI and SpeI sites.

<u>Acu II</u>	<u>Nco I</u>	<u>Spe I</u>	<u>Eco RI</u>
TTCGAA	ACC	ATGGCCGGGACTAGT	GGC.CAC.CAT.CAC.CAT.CAC.CAT.TAA.CGGAATTC
Thr . Ser . Gly .	His . His . His . His . His . His .		

The amino acid sequence of Figure 3 relates to Seq. ID no. 27 and the nucleic acid sequence of Figure 3 relates to Seq. ID. No. 26.

FIG. 4 SDS-PAGE: Nef-Tat-his fusion protein



- 1: MW (175/83/62,5/47, 5/32,5/25/16,5/6,5 kDa
- 2: TNH/23 SP eluate (250 ng)
- 3: TNH/23 Purified bulk (250 ng)
- 4: TNH/22 Purified bulk (250 ng)
- 5: MW (175/83/62,5/47, 5/32,5/25/16,5/6,5 kDa
- 6: TNH/23 SP eluate (400 ng)
- 7: TNH/23 Purified bulk (400 ng)
- 8: TNH/22 Purified bulk (400 ng)

Daiichi Silver Staining



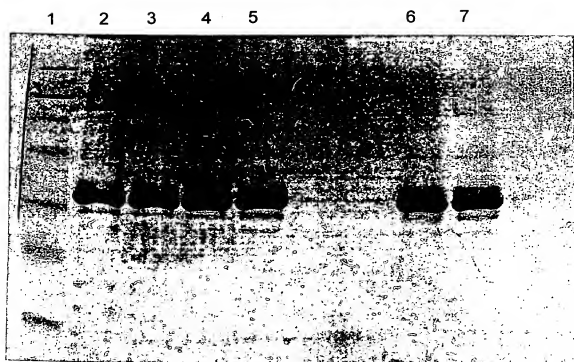
Blot α Nef-Tat (LAS 97340)

1 2 3 4



Blot Tat2

FIG. 5 SDS-PAGE: Nef-Tat-his fusion protein



Coomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
- 2: TNH/23 SP eluate (4 μ g)
- 3: TNH/23 Superdex200 eluate (4 μ g)
- 4: TNH/23 Purified bulk (4 μ g)
- 5: TNH/22 Purified bulk (4 μ g)
- 6: TNH/23 Purified bulk (4 μ g) / non reducing conditions
- 7: TNH/22 Purified bulk (4 μ g) / non reducing conditions

FIG. 6A Tat-specific antibody titers and isotypes

group	immunization	midpoint titers					ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b		
1	oxydized Tat	353557	135538	98771	98763	1.372	
2	reduced Tat	252275	72087	76273	72014	0.945	
3	oxydized Nef-Tat	246466	179616	60835	53563	2.953	
4	reduced Nef-Tat	91726	73767	30948	20679	2.384	
5	adjuvant only	<4000	<4000	<4000	<4000		

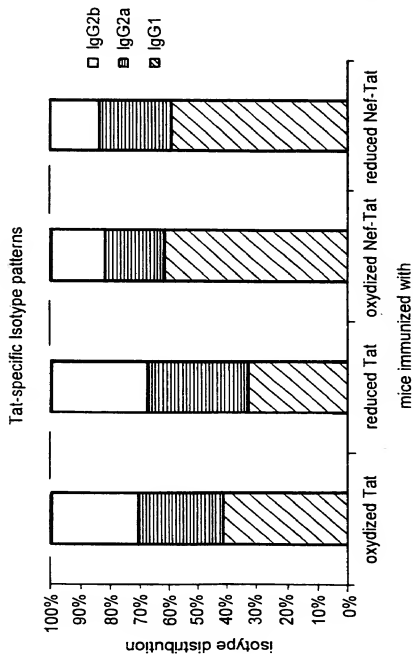


FIG. 6B Tat-specific antibody titers and isotypes

group	immunization	midpoint titers				ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b	
1	reduced Tat	212789	123242	62697	55763	1.966
2	reduced Nef-Tat	75676	84046	18449	11692	4.556
3	adjuvant only	<4000	<4000	<4000	<4000	

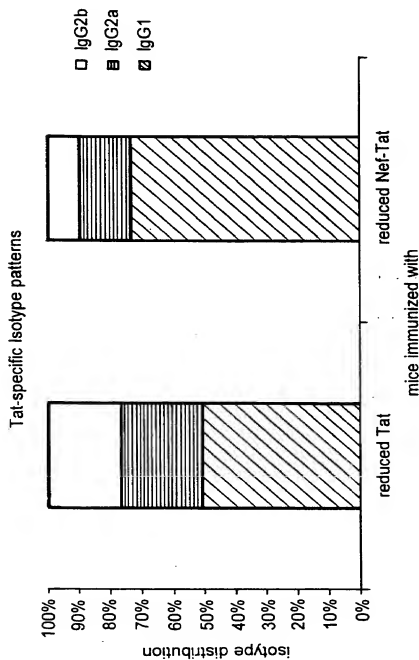


FIG. 7

Antigen-specific lymphoproliferative response of pooled lymph node cells

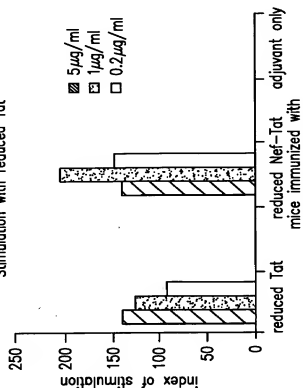
(3H) Thymidine incorporation in cpm

	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat	41967	18511	789
5 μ g/ml	37609	32346	415
1 μ g/ml	27640	23408	397
0.2 μ g/ml			
reduced Nef-Tat	43882	31694	483
5 μ g/ml	33865	28094	245
1 μ g/ml	25079	22891	383
0.2 μ g/ml			
medium	300	161	571

Data expressed as stimulation index

	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat	140	115	1
5 μ g/ml	125	201	1
1 μ g/ml	92	145	1
0.2 μ g/ml			
reduced Nef-Tat	146	197	1
5 μ g/ml	113	174	0
1 μ g/ml	84	142	1
0.2 μ g/ml			
medium	1	1	1

Stimulation with reduced Tat



Stimulation with reduced Nef-Tat

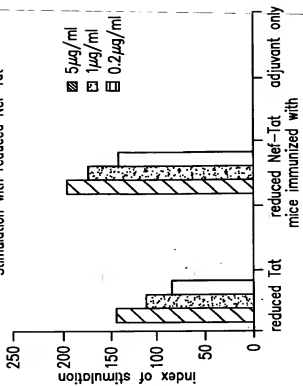


FIG. 8 Cell binding assay

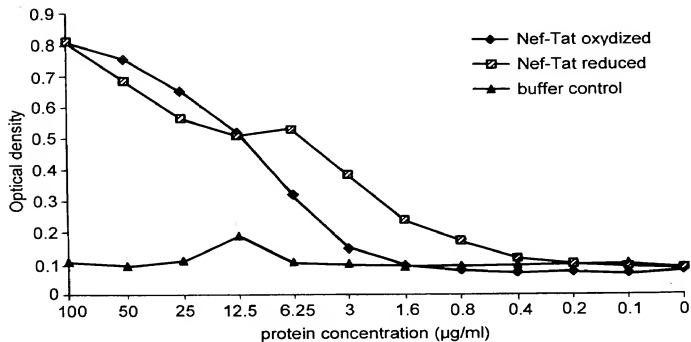
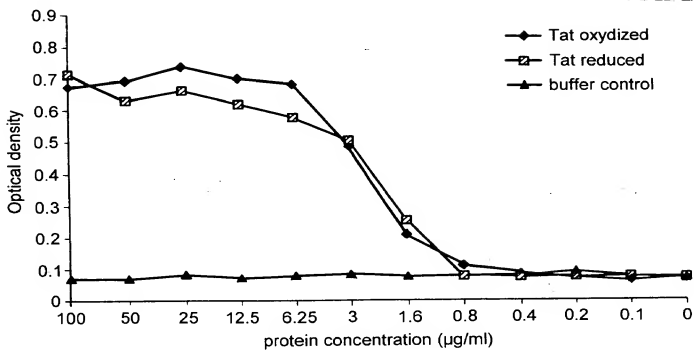


FIG. 9 Inhibition of cell growth

